

A New Polyclad Flatworm, *Idiostylochus tortuosus* gen. nov., sp. nov. (Platyhelminthes, Polycladida) from France. Can this Foreign Flatworm be Responsible for the Deterioration of Oyster and Mussel Farms?

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A new species of polyclad flatworm, *Idiostylochus tortuosus* gen. nov., sp. nov. (Polycladida, Idioplanidae), from Arcachon Bay (France) is described. This description is based on a morphological analysis and a molecular analysis using partial sequences of the 28S and cytochrome Oxidase I (*COI*) genes. After the molecular analysis *Idiostylochus* gen. nov. appears to be the second genus of the Family Idioplanidae and closely related to the family Latocestidae as well as the genera *Leptostylochus* and *Mirostylochus*. The molecular data revealed that the new species may belong to an Indonesian or Indo-Pacific family, closely related to genera with origins in South Pacific Ocean waters. This species was found feeding on the oysters and mussels of the Arcachon farms.

Key words: Acotylea, Idioplanidae, Non-native species, *Magallana gigas*, Oyster culture.

BACKGROUND

France is the main producer and consumer of oysters in Europe. French oyster farms produce around 80,000 tons of Japanese oysters (*Magallana gigas* (Thunberg, 1793), formerly *Crassostrea gigas*) per year, representing 78% of European annual production (FAO 2021). With an annual production, in normal conditions, of around 10,000 tons of *M. gigas* (Vieira

et al. 2020), Arcachon Bay is not only one of the most important areas for French oyster farming, it is also one of the first areas to have implemented this type of culture (Bouchet et al. 1997; Buestel et al. 2009). Over the years, three different species of oysters have been farmed in Arcachon Bay: the European flat oyster (*Ostrea edulis* Linnaeus, 1758), the Portuguese cupped oyster (*Crassostrea angulata* (Lamarck, 1819)), and the above-mentioned Japanese oyster. The appearance of different

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epizootics caused the collapse of the European and Portuguese species. At present, French oyster farmers focus their cultures almost exclusively on *M. gigas*. This species was introduced to Arcachon in the 1970s to gradually replace the declining oyster cultures of *C. angulata* (Buestel et al. 2009). Arcachon Bay benefited in May 1971 from an initial input of 52.5 tonnes of broodstock from British Columbia in Canada (very large oysters about 10 years old), reinforced by 60 tonnes in 1972 and 25 tonnes in 1973, making a total of 137.5 tonnes of mother oysters that were placed in several reserves along the Basin. At the same time, large quantities of spat from Japan were introduced (Grizel and Héral 1991). *Magallana gigas* is characterized by its large size, rapid growth and high tolerance to environmental changes compared to other oyster species (FAO 2005–2021).

Since the introduction of *Magallana gigas* from Japan, Canada and North America in the 1970s (Grizel and Héral 1991), several non-indigenous species have been observed (e.g., Bachelet et al. 2009; Lavesque et al. 2013; Gouillieux and Massé 2019), introduced directly from the Pacific or during regular transports of batches between French oyster farming areas. The description of a new species in the Arcachon Bay, which ultimately turned out to be a non-indigenous species, occurred recently with the annelid polychaete *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (Annelida) (Lavesque et al. 2020).

In 2020, the presence of large amounts of unknown polyclads (Platyhelminthes, Polycladida) feeding on individuals of *M. gigas* and *Mytilus* sp. were recorded in Arcachon Bay. The occurrence of this polyclad may be related to the increasing oyster mortality recorded since 2019 in oyster farms (Vieira et al. 2020) and mussel beds in Arcachon Bay (local marine fisheries committee, pers. com.)

Most polyclads are predators of other small invertebrates, such as crustaceans, ascidians, cnidarians, gastropods, or bivalves (Barton et al. 2020; Jennings 1957; Newman and Cannon 2003; Lee 2006; Teng et al. 2022). At present, the suborder Acotylea (Polycladida) is divided into three superfamilies: Discoceloidea Dittmann, Cuadrado, Aguado, Noreña and Egger, 2019; Leptoplanoidea Faubel, 1984; and Stylochoidea Poche, 1926.

Within Acotylea, known predators of bivalves belong mainly to the superfamily Stylochoidea. They prey on mussels (Galleni et al. 1980), scallops (Heasman et al. 1998), giant clams (Newman et al. 1993) and oysters (Danglade 1919; Pearse and Wharton 1938; Littlewood and Marsbe 1990; Newman et al. 1993). Although the mechanism used to open the valves of the prey varies among species, this process usually

starts with the polyclad gliding over the posterior end of the valves, followed by the secretion of a considerable amount of mucus. This secretion could serve to immobilize the prey (Hyman 1951), as well as to avoid desiccation and potential attacks by other predators (Gammoudi et al. 2017). Once in position, the polyclad inserts its pharynx into the bivalve, damages the adductor muscle to prevent the prey from closing its valves, and digests it (Gammoudi et al. 2017).

The aim of this study is to describe and determine the systematic position of a new species of Polycladida found in Arcachon Bay. These animals are known as predators of bivalve mollusks, and the molecular evidence suggests that they are a non-native species. These preliminary results will guide future studies focused on the habitat, ecology and impact of this species in France.

MATERIALS AND METHODS

Sampling

The specimens were collected by hand in the Arcachon Bay (French Atlantic Coast) (Fig. 1) in October 2020. The live specimens were anesthetized with a solution of 7% MgCl₂ and photographed. A small sample of tissue from the lateral margin was preserved in absolute ethanol for DNA extraction. The rest of the specimens were fixed individually in frozen 10% formalin buffered with filtered seawater. After 24 h, the worms were transferred to 30% ethanol for an hour, then to 50% ethanol for an hour and finally stored in 70% ethanol.

Histological processing

The fixed specimens were dehydrated in progressive ethanol solutions, embedded in Paraplast, and sectioned sagittally at 10 µm. The sections were stained with the Azan trichrome stain. To identify the species, reconstructions of the internal anatomy of the reproductive system were performed using a Zeiss Axio Scope A1 microscope.

DNA extraction, amplification and sequencing

DNA from four individuals (Table 1) was extracted using the phenol-chloroform protocol (Chen et al. 2010), using the tissue samples fixed in absolute ethanol. Once the DNA was extracted, its purity and concentration were calculated with a NanoDrop spectrophotometer (Thermo Fisher Scientific).

Two partial sequences, one of approximately

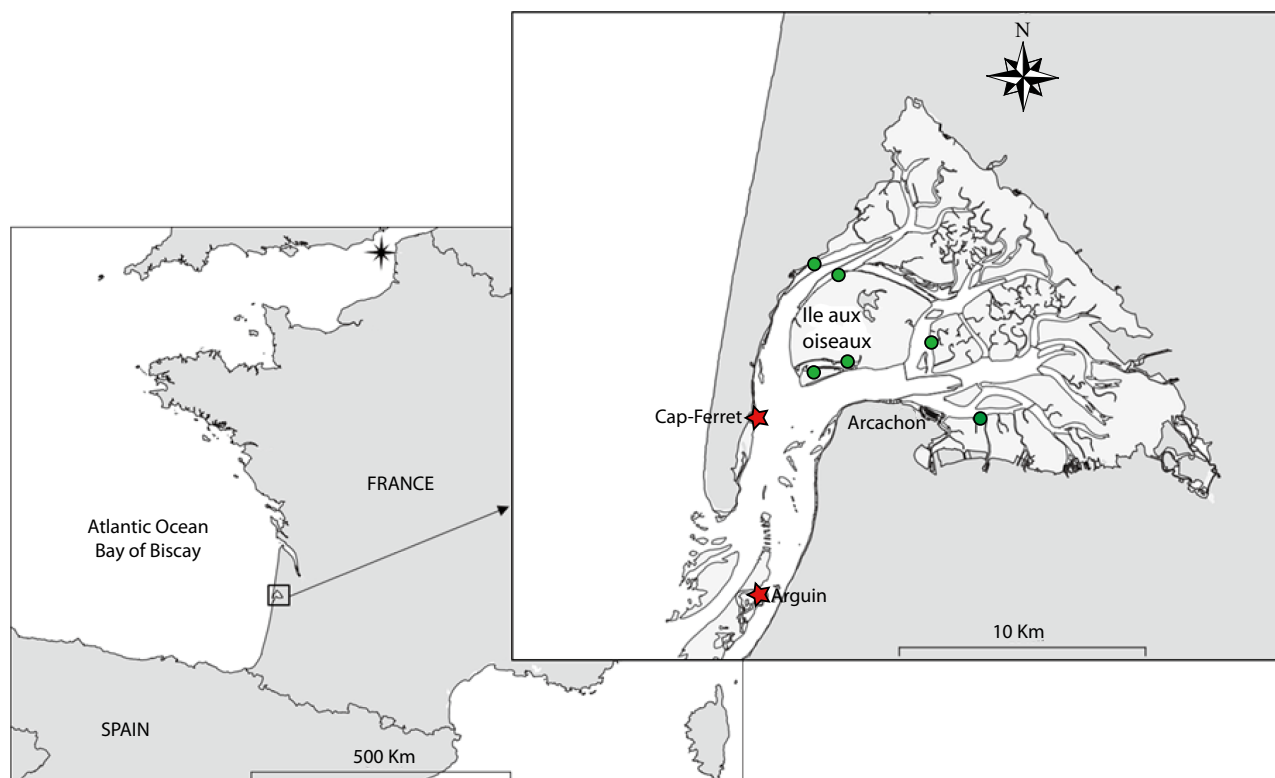


Fig. 1. Bay of Archachon. Sampling sites. The green dots show the localities inside the bay, the red stars mark out the localities outside the bay.

Table 1. List of species included in the molecular analysis with their respective locality, GenBank accession number or museum catalogue number (MNCN) and reference

Family	Species	28S	COI	Locality	Reference
Discoceloidea					
Cryptocelidae	<i>Phaenocelis medvedica</i> Marcus 1952	KY263701	-	Brazil	Bahia et al. 2017
	<i>Phaenocelis peleca</i> Marcus and Marcus, 1968	MH700342	-	Colombia	Litvaitis et al. 2019
	<i>Phaenocelis purpurea</i> (Schmarda, 1859)	MH700347	-	Curazao	Litvaitis et al. 2019
Discocelidae	<i>Adenoplana evelinae</i> Marcus, 1950	MH700268	-	Panama	Litvaitis et al. 2019
	<i>Discocelis tigrina</i> (Blanchard, 1847)	MN384690	MF993332	Spain	Dittmann et al. 2019; Kenny et al. 2019
	<i>Discocelis</i> sp.	LC508146	LC508206	Japan	Oya and Kajihara 2020
Ilyplanidae	<i>Ilyella gigas</i> (Schmarda, 1859)	LC508139	LC508199	Japan	Oya and Kajihara 2020
	<i>Tripylocelis typica</i> Haswell, 1907	MW377505	-	Australia	Rodríguez et al. 2021
	<i>Zyganroides serpulidicola</i> Oya, Tsuyuki and Kajihara, 2020	-	LC528161	Japan	Oya et al. 2020
Leptoplanoidea					
Leptoplanidae	<i>Leptoplana tremellaris</i> (Müller OF, 1773)	KY263695	-	Spain	Bahia et al. 2017
Notocomplanidae	<i>Notocomplana hagiyai</i> Oya and Kajihara, 2017	LC508129	LC176003	Japan	Oya and Kajihara 2020; Oya and Kajihara 2017
	<i>Notocomplana humilis</i> (Stimpson, 1857)	LC100085	LC508204	Japan	Tsunashima et al. 2017; Oya and Kajihara 2020
	<i>Notocomplana japonica</i> (Kato, 1937)	LC100087	LC176018	Japan	Tsunashima et al. 2017; Oya and Kajihara 2017
	<i>Notocomplana koreana</i> (Kato, 1937)	LC100086	LC176014	Japan	Tsunashima et al. 2017
	<i>Notocomplana septentrionalis</i> (Kato, 1937)	LC508130	LC176028	Japan	Oya and Kajihara 2020; Tsunashima et al. 2017
Notoplanidae	<i>Notoplana australis</i> (Schmarda, 1859)	AY157153	-	Australia	Lockyer et al. 2003
	<i>Notoplana delicata</i> Yeri and Kaburaki, 1918	LC100088	LC508205	Japan	Tsunashima et al. 2017; Oya and Kajihara 2020

Table 1. (Continued)

Family	Species	28S	COI	Locality	Reference	
Pseudostylochidae	<i>Pseudostylochus elongatus</i> (Kato, 1937)	LC100083	LC508207	Japan	Tsunashima et al. 2017; Oya and Kajihara 2020	
	<i>Pseudostylochus intermedius</i> Kato, 1939	LC508141	LC508201	Japan	Oya and Kajihara 2020	
	<i>Pseudostylochus obscurus</i> (Stimpson, 1857)	LC100084	LC508197	Japan	Tsunashima et al. 2017; Oya and Kajihara 2020	
	<i>Pseudostylochus takeshitai</i> (Yeri and Kaburaki, 1918)	LC508142	LC508202	Japan	Oya and Kajihara 2020	
Stylochoplanidae	<i>Amemiyaia pacifica</i> Kato, 1944	LC508143	LC508203	Japan	Oya and Kajihara 2020	
	<i>Armatoplana leptalea</i> (Marcus, 1947)	KY263648	-	Brazil	Bahia et al. 2017	
	<i>Comoplana pusilla</i> (Bock, 1924)	LC508134	LC508194	Japan	Oya and Kajihara 2020	
	<i>Stylochoplana</i> sp.	-	KP259873	New Zealand	Salvitti et al. 2015	
Stylochoidea	Hoploplanidae	<i>Hoploplana californica</i> Hyman, 1953	KC869850	-	USA	Laumer and Giribet 2014
		<i>Hoploplana divae</i> Marcus, 1950	KY263692	-	Brazil	Bahia et al. 2017
	<i>Hoploplana elisabelloi</i> Noreña, Rodríguez, Pérez and Almón, 2015	-	KT363735	-	-	Aguado et al. 2016
	<i>Hoploplana ornata</i> Yeri and Kaburaki, 1918	LC508135	LC508195	Japan	Oya and Kajihara 2020	
	<i>Hoploplana villosa</i> (Lang, 1884)	LC100076	-	Japan	Tsunashima et al. 2017	
	Idioplanidae	<i>Idioplana atlantica</i> (Bock, 1913)	MH700310	-	Panama	Litvaitis et al. 2019
		<i>Idioplana australiensis</i> Woodworth, 1898	MW377499	MW375903	Australia	Rodríguez et al. 2021
		<i>Idiostylochus tortuosus</i> sp. nov.	OM367916	OM365887	France	This study
		<i>Idiostylochus tortuosus</i> sp. nov.	OM367917	OM365888	France	This study
		<i>Idiostylochus tortuosus</i> sp. nov.	OM367918	OM365889	France	This study
<i>Idiostylochus tortuosus</i> sp. nov.		OM367919	ON796529	France	This study	
<i>Idiostylochus tortuosus</i> sp. nov.	ON796524	ON796527	France	This study		
<i>Idiostylochus tortuosus</i> sp. nov.	ON796525	ON796528	France	This study		
<i>Idiostylochus tortuosus</i> sp. nov.	ON796526	ON796530	France	This study		
Latocestidae	<i>Eulatocestus australis</i> Rodríguez, Hutchings and Williamson, 2021	MW377502	MW375906	Australia	Rodríguez et al. 2021	
	<i>Latocestus plehni</i> Laidlaw, 1906	MK299376	-	Canary Islands	Cuadrado et al. 2021	
	<i>Latocestus plehni</i> Laidlaw, 1906	MK299377	-	Cabo Verde	Cuadrado et al. 2021	
Planoceridae	<i>Heteroplanocera katoi</i> Oya and Kajihara, 2021	LC545568	LC545561	Japan	Oya and Kajihara 2021	
	<i>Paraplanocera oligoglana</i> (Schmarda, 1859)	LC545569	LC545563	Japan	Oya and Kajihara 2021	
	<i>Paraplanocera</i> sp.	KY263699	-	Greece	Bahia et al. 2017	
	<i>Planocera multi tentaculata</i> Kato, 1944	LC100081	LC508192	Japan	Tsunashima et al. 2017; Oya and Kajihara 2020	
Plehnidae	<i>Planocera reticulata</i> (Stimpson, 1855)	LC508148	LC508208	Japan	Oya and Kajihara 2020	
	<i>Paraplehnia pacifica</i> (Kato, 1939)	LC508132	LC508193	Japan	Oya and Kajihara 2020	
	<i>Paraplehnia seisuiiae</i> Oya, Kimura and Kajihara, 2019	LC467000	LC466999	Japan	Oya et al. 2019	
Stylochidae	<i>Leptostylochus gracilis</i> Kato, 1934	LC100078	-	Japan	Tsunashima et al. 2017	
	<i>Leptostylochus</i> cf. <i>gracilis</i> Kato, 1934	LC508138	LC508198	Japan	Oya and Kajihara 2020	
	<i>Leptostylochus victoriensis</i> Beveridge, 2018	MW377495	MW375899	Australia	Rodríguez et al. 2021	
	<i>Mirostylochus akkeshiensis</i> Kato, 1937	LC508149	LC508209	Japan	Oya and Kajihara 2020	
	<i>Stylochus</i> cf. <i>aomori</i> Kato, 1937	LC508140	LC508200	Japan	Oya and Kajihara 2020	
	<i>Stylochus ijimai</i> Yeri & Kaburaki, 1918	LC100079	-	Japan	Tsunashima et al. 2017	
	<i>Stylochus neapolitanus</i> (Delle Chiaje, 1841)	MZ292841	-	Spain	Rodríguez et al. Unpublished	
	<i>Stylochus refertus</i> Du Bois-Reymond Marcus, 1965	KY263694	-	Brazil	Bahia et al. 2017	
	<i>Stylochus zebra</i> (Verrill, 1882)	AF342800	-	USA	Mallatt and Winchell 2002	
	<i>Stylochus</i> sp.	KY263743	-	Peru	Bahia et al. 2017	
Outgroup						
Cestoplanidae	<i>Cestoplana nopperabo</i> Oya and Kajihara, 2018	LC322284	LC322283	Japan	Oya and Kajihara 2018	
Pericelidae	<i>Pericelis flavomarginata</i> Tsuyuki, Oya, Jimi and Kajihara, 2020	LC568535	LC568538	Japan	Tsuyuki et al. 2020	

1,000 bp from the 28S gene and another of 700 bp from the *COI* gene, were amplified by PCR. For the 28S sequences, a forward primer (5'-AGCCCAGCACCGAATCCT-3') and a reverse primer (5'-GCAAACCAAGTAGGGTGTGCGC-3') were used (Cuadrado et al. 2021). The reaction was carried out in a final volume of 25 µl with 1 µl of DNA, 12.5 µl of DreamTaq DNA polymerase and 1 µl of each primer. The amplification protocol used was an initial denaturation step at 95°C (4 min), followed by 35 cycles of denaturation at 95°C (1 min), annealing at 59°C (1 min) and extension at 72°C (1 min), with a final extension at 72°C (10 min).

For the *COI* sequences, the primers Acotylea_COI_F (5'-ACTTTATTCTACTAATCATAAGGATATAGG-3') and Acotylea_COI_R (5'-CTTTCCTCTATAAAATGTTACTATTTGAGA-3') were used (Oya and Kajihara 2020). The reaction was carried out with the same volumes as those used for the 28S gene. The amplification protocol was as follows: initial denaturation step at 94°C (5 min), 35 cycles of denaturation at 94°C (30 s), annealing at 50°C (30 s) and extension at 72°C (1 min), with a final extension at 72°C (7 min).

The PCR products were purified using ExoSAP (Bell 2008). The purified samples were sent to Secugen S.L. (www.secugen.es) for sequencing. Finally, the sequences obtained from the forward and reverse primers were combined and edited with Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA; http://www.genecodes.com).

Sequence alignment and molecular analyses

The different trees were obtained applying the Maximum Likelihood (ML) and Bayesian Inference (BI) methods, using the sequences obtained in this work and those available in GenBank (NCBI) of representative species of the main families of the suborder Acotylea (Table 1). Two Cotylea were used as outgroups: *Pericelis flavomarginata* Tsuyuki, Oya, Jimi and Kajihara, 2020 and *Cestoplana nopperabo* Oya and Kajihara, 2018 (Table 1). Sequence alignment was performed with MAFFT (Katoh et al. 2018) with the default options. Ambiguous regions were removed using Gblocks ver. 0.91b (Talavera and Castresana 2007) with the least restrictive options. The alignments were checked manually with BioEdit (Hall 1999).

For this study, a dataset of 706 bp and 58 sequences for the 28S gene analysis and a dataset of 640 bp and 40 sequences for the *COI* gene analysis were used (the complete sequences of the new species can be found in GenBank, Table 1). The substitution model used for all the analyses was GTR+I+G, which

was determined with ModelFinder (Kalyaanamoorthy et al. 2017) using the Akaike Information Criterion (AIC) (Akaike 1974).

ML analyses were performed with IQ-TREE (Trifinopoulos et al. 2016). Nodal support was calculated with a bootstrap standard test with 1,000 replicates. BI analyses were performed with MrBayes 3.2.3 (Ronquist et al. 2012). Two simultaneous analyses of 10,000,000 generations were run with four chains (one cold, three heated) and a tree sampling frequency of 1,000. The convergence of the chains was determined using the value of the standard deviation of the frequencies (< 0.05). The first 25% of the trees were discarded as burn-in. The trees resulting from both methods were visualized and edited with iTOL ver. 6.3 (Letunic and Bork 2021).

RESULTS

SYSTEMATICS

Order Polycladida Lang, 1881
Suborder Acotylea Lang, 1884
Superfamily Stylochoidea Poche, 1926
Family Idioplanidae Dittmann, Cuadrado,
Aguado, Noreña and Egger, 2019

Idiostylochus gen. nov.

urn:lsid:zoobank.org:act:B1874F30-7A5B-45C6-AD8A-34A37FC66E5A

Diagnosis: Idioplanidae with the pharynx in the middle of the body. Cerebral and marginal eyes present. Male copulatory apparatus with spermiducal bulbs and prostatic vesicle. Seminal vesicle absent. Female apparatus with a tubular Lang's vesicle and cement glands. The vagina makes a posterior turn before reaching the penis papilla.

Type species: *Idiostylochus tortuosus* sp. nov.

Etymology: The name *Idiostylochus* derives from a combination of *Idioplana* and *Stylochus*, regarding the presence of a unique combination of characters found in part in these genera.

Idiostylochus tortuosus sp. nov.

(Fig. 2)

urn:lsid:zoobank.org:act:BCF1166D-6D0A-4B79-899A-59D3B262644C

Type material: Holotype: 1 specimen. Arcachon Bay, France, October 20, 2020. Sagittal sections stained with Azan trichrome. MNCN 4.01/4263 to MNCN 4.01/4291 (29 slides). GeneBank accession numbers:

ON796526 (28S), ON796530 (COI).

Paratype: 1 specimen. Arcachon Bay, France, October 20, 2020. Sagittal sections stained with Azan trichrome. MNCN 4.01/4292 to MNCN 4.01/4335 (44 slides).

Additional material: tissues preserved in 100% Ethanol and sagittal sections stained with Azan trichrome; Arcachon Bay, France, October 20, 2020.

For GeneBank accession numbers see table 1.

Diagnosis: Male copulatory apparatus with conspicuous spermiducal bulbs and a small prostatic vesicle. Elongated male atrium covered by glandular tissue. Female apparatus with a vagina bulbosa and well-developed cement glands that surround the two sections of the vagina (externa e interna).

Etymology: The specific name derives from the Latin tortuosus, due to the winding and complex course of the distal portion of the vasa deferentia and spermiducal bulbs.

Description: Body shape rounded-oval with slightly undulated margins (Fig. 2A). Holotype 1.8 cm long and 1.1 cm wide, paratype 2.2 cm long and 1.5 cm wide. Body consistency firm and fleshy, more delicate and thinner towards the margins. Tentacles lacking. Cerebral and marginal eyes present. Background pigmentation chocolate brown to caramel in the margins. Numerous dark spots scattered over the dorsal surface, more abundant along the main body axis (Fig. 2A). Ventral surface pale, with grey to beige tonalities. Epithelium, basal membrane and body musculature more developed on the dorsal than on the ventral side (Fig. 2B). Pharynx ruffled, well developed, extends throughout the mid-body region. Oral pore at the beginning of the posterior body-half.

Reproductive system: male and female reproductive organs are located directly after the pharynx, in the posterior half of the animal. Male copulatory apparatus consisting of a conical penis papilla (or penial bulb) and a small pyriform prostatic vesicle, seminal vesicle absent (Fig. 2C, E). Vasa deferentia forms bulky spermiducal bulbs. The diameter of the bulbs decreases as they approach each other until they join forming the common vas deferens, which opens into the middle region of the ejaculatory duct. Both vasa deferentia and spermiducal bulbs follow a tortuous course. Free prostatic vesicle. Male atrium elongated and covered with glandular, spongy tissue.

Female gonopore posterior to the male gonopore (Fig. 2D, E, F). With vagina bulbosa. The vagina externa curves anteriorly to the male reproductive system, then upwardly and continues posteriorly into the vagina interna. The oviduct opens between the vagina externa and interna. Connected to the vagina interna is an elongated and tubular Lang's vesicle (Fig. 2D, E).

The general appearance of the female apparatus is compact with small visible folds, mainly in the vagina externa, and surrounded by abundant cement glands.

Biology and occurrence

Idiostylochus tortuosus was found in oyster cultures of *Magallana gigas*. Individuals were collected living in the mantle cavity of diseased or dead oysters as well as swimming around the oyster farming devices. Some individuals have also been observed feeding on natural beds of *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 near the oyster farms (Vieira and Nowaczyk pers. com.). Although the presence of *Idiostylochus* was known long ago by oyster farmers, in recent years, the frequency and number of polyclads specimens seem to have increased, and their presence has caused noticeable damage to oyster and mussel crops.

Taxonomical remarks

The superfamily Stylochoidea, where the new species *Idiostylochus tortuosus* was placed, presents a free prostatic vesicle (Faubel 1983). From the molecular point of view, the closest related genera (see - tree 28S, Fig. 4) are *Idioplana* Woodworth, 1898 (Idioplanidae), *Leptostylochus* Bock, 1925 (Stylochidae), and also, but less related, *Mirostylochus* Kato, 1937 (Stylochidae), *Latocestus* Plehn, 1896 and *Eulatocestus* Faubel, 1983 (Latocestidae). All these genera present a free prostatic vesicle and either developed spermiducal bulbs or an elongated seminal vesicle. The new species shares some characters with these genera, while others are clearly different. A comparative discussion follows.

Leptostylochus (Stylochidae) (Fig. 3A) is characterized by an elongated slender body shape; tentacular, cerebral, marginal and often frontal eyes; male copulatory apparatus with an unarmed penis papilla and without seminal vesicle, but with spermiducal bulbs that join into a common vas deferens before entering the medial region of the ejaculatory duct; female reproductive system with a developed Lang's vesicle (Kato 1934; Faubel 1983; Beveridge 2017). *Idiostylochus* gen. nov. resembles *Leptostylochus* because of the presence of large spermiducal bulbs. Furthermore, the female system shows common features, like the well-developed shell glands around the vagina externa. In contrast, the Lang's vesicle is conspicuous in *Leptostylochus* and reduced in *Idiostylochus*, which appears as a small tubular duct.

Latocestus and *Eulatocestus* (Latocestidae) share a similar morphology. Both genera have spermiducal bulbs, unarmed penis papilla and a

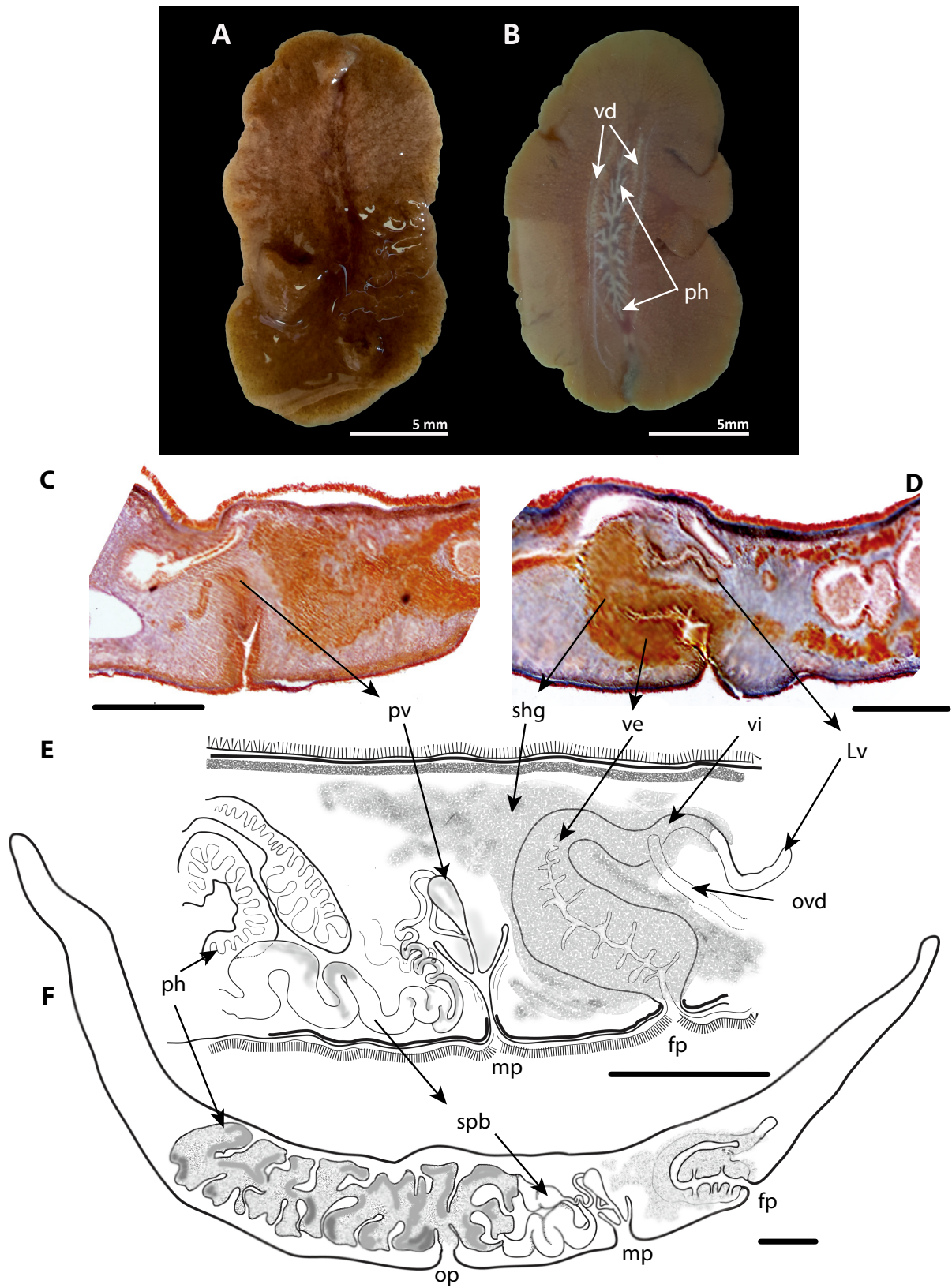


Fig. 2. *Idiostylochus tortuosus* gen. nov., sp. nov. A–B, Live specimen. A, dorsal view; B, ventral view. C–D, histological section through male (left) and female (right) copulatory organs. E, sagittal reconstruction of the reproductive system. F, sagittal reconstruction of whole animal (anterior end at the left). Scale bars = 500 μ m.

simple female apparatus with Lang's vesicle (Plehn 1896; Faubel 1983) (Fig. 3B). The main difference between *Latocestus* and *Eulatocestus* is the lining of the prostatic vesicle: irregular or fingered in *Latocestus* and a web of glandular follicles in *Eulatocestus*. Both genera are distinguishable from *Idiostylochus* by the morphology of the female apparatus. The presence of a well-developed Lang's vesicle and a simple, non-bulbous vagina with much less abundant cement glands differentiates both genera from *Idiostylochus*.

Idioplana (Idioplanidae) is characterized by a male copulatory organ with an unarmed penis papilla, prostatic and seminal vesicle; female apparatus extended over the male apparatus; and an anchor-shaped Lang's vesicle (Woodworth 1898; Meixner 1907; Faubel 1983; Rodríguez et al. 2021) (Fig. 3C).

The analysis of the morphological data reveals external anatomical similarities, such as the oval body shape, the reddish-brown dorsal and whitish ventral coloration and the arrangement of the cerebral eyes, but both genera are clearly differentiated by the presence of conspicuous tentacles in *Idioplana*, absent in *Idiostylochus*, and the differences between the reproductive systems. In *Idiostylochus* the seminal vesicle is absent, the female canal is shorter, since the vagina interna do not extend anteriorly over the male copulatory organ, and the Lang's vesicle is tubular.

Within the male apparatus, there are clear differences. In *Idioplana* a seminal vesicle is present, while in *Idiostylochus* is absent. In *Idiostylochus* the function of the seminal vesicle is carried out by the spermiducal bulbs, since the latter replace the former in its absence.

Although the molecular analysis clusters *Mirostylochus* (Stylochidae) near *Idiostylochus*, the two genera are morphologically distinct. *Mirostylochus* is characterized by tentacular, cerebral and marginal eyes; and a female apparatus with ductus vaginalis, from which the vagina interna opens to the exterior behind the female gonopore. Lang's vesicle is absent (Kato 1937; Tokinova 2003). *Idiostylochus* gen. nov. and *Mirostylochus* differ in the morphology of the female apparatus, as *Idiostylochus* possesses a tubular Lang's vesicle and lacks ductus vaginalis.

Molecular approach

The molecular studies are based on two datasets of the genes 28S (nuclear) and *COI* (mitochondrial). The methods applied for the analyses were Maximum Likelihood (ML) and Bayesian Inference (BI).

Within the tree generated during the analysis of 28S (Fig. 4) we can distinguish three well-supported branches, the clusters of the superfamilies Discoceloidea, Leptoplanoidea and Stylochoidea

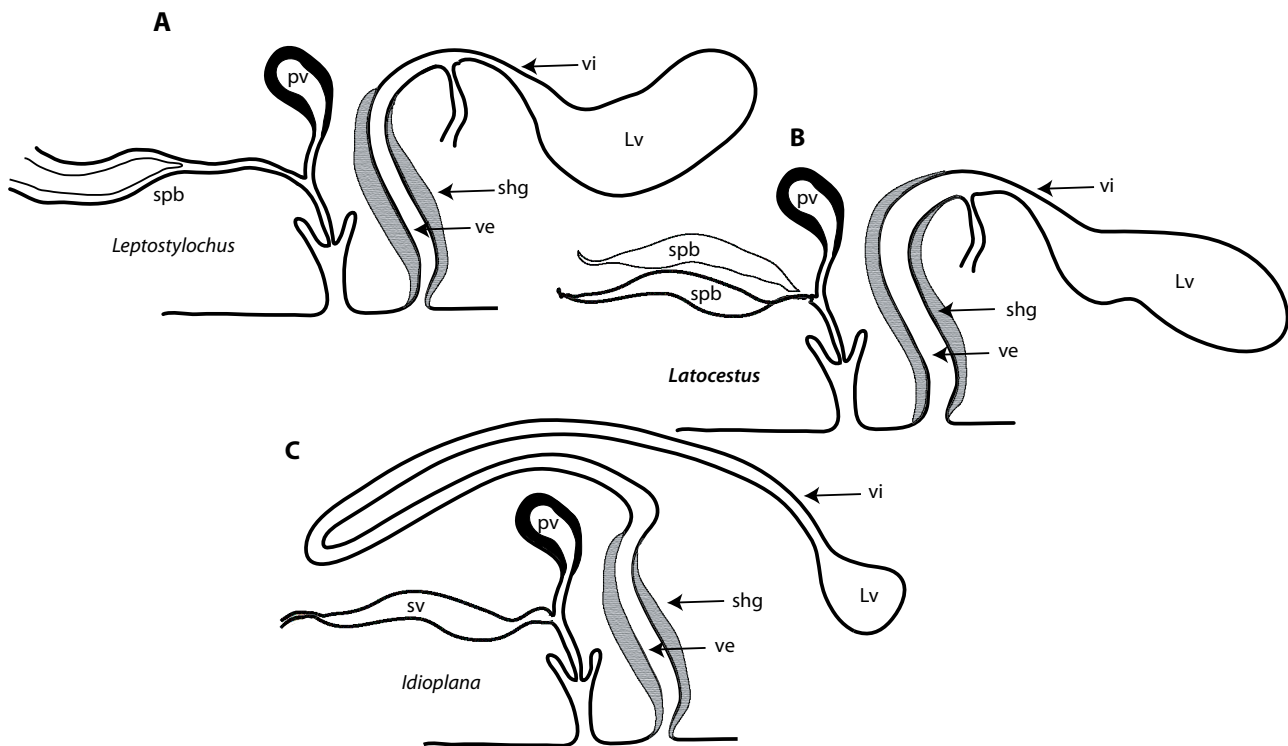


Fig. 3. Comparison of the copulatory apparatuses of different genera of Stylochoidea. A, *Leptostylochus* Bock, 1925; B, *Latocestus* Plehn, 1896; C, *Idioplana* Woodworth 1898.

Tree scale: 0.1

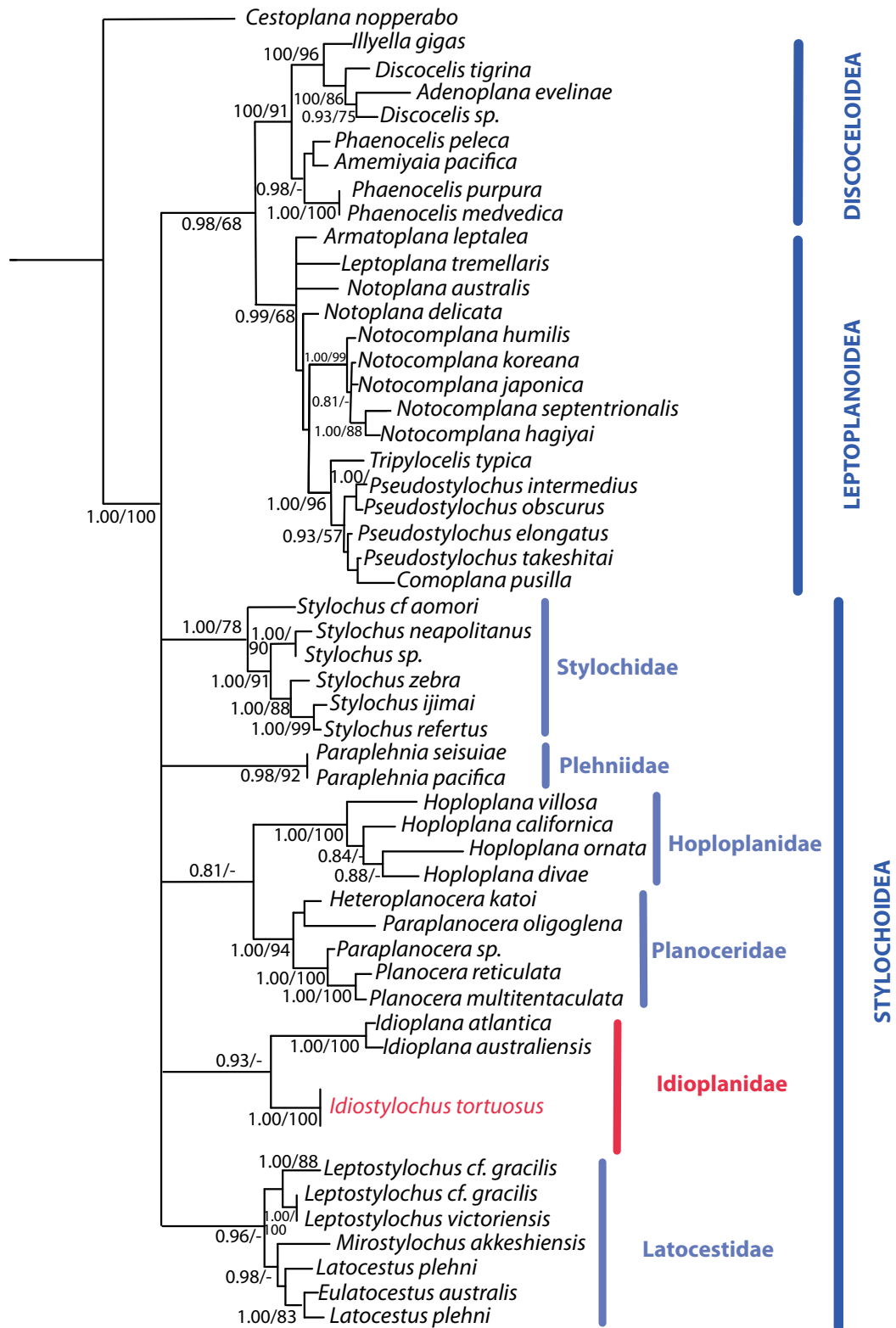


Fig. 4. Bayesian / Maximum likelihood tree based on partial sequences of the 28S gene (total length: 706 bp). Numbers in the nodes correspond to the posterior probability values of the BI analysis (> 0.70) and bootstrap support values of the ML analysis (> 50), respectively.

respectively. The evidence provided by the 28S gene allows us to cluster the genera that are phylogenetically more related at the superfamily level within Acotylea. In our analysis, which is focused on determining the systematic position of the new species, *Idiostylochus tortuosus*, we found that the species is closely related to *Idioplana atlantica* and *I. austaliensis*, both of which belong to the Family Idioplanidae. On the other hand, the genera *Mirostylochus* and *Leptostylochus* do not cluster within the Family Stylochidae but with *Latocestus* and *Eulatocestus*, the selected representatives of the family Latocestidae in this study.

Other genera used in the 28S gene analysis are grouped in the same families as in previous molecular and morphological analyses carried out by previous authors (Dittmann et al. 2019; Litvaitis et al. 2019; Oya and Kajihara 2020).

In the *COI* analysis (Fig. 5) we have only taken into account values > 70 (ML), although we show values between < 70 and > 60 as they reflect a certain relationship among species or genera. Therefore, our

analyses show strongly supported species within a genus, while low values at the family level are meaningless.

DISCUSSION

Currently, the presence of *Idiostylochus tortuosus* sp. nov. has been confirmed in the oyster farms of Arcachon Bay (Atlantic basin, France) and is also likely to be in the Etang de Thau (Mediterranean basin, France) associated with oyster mortalities (Vieira et al. 2020).

Through the results obtained with the *COI* mitochondrial gene (Fig. 5) and 28S nuclear gene analysis (Fig. 4), three goals of this study have been achieved: the establishment of *Idiostylochus tortuosus* as a new species, the evidence of the close relation of *Idiostylochus* with species from the Pacific Ocean and the new combination of genera in some known families.

Therefore, together with the morphological evidence, *Idiostylochus* can be included with *Idioplana*

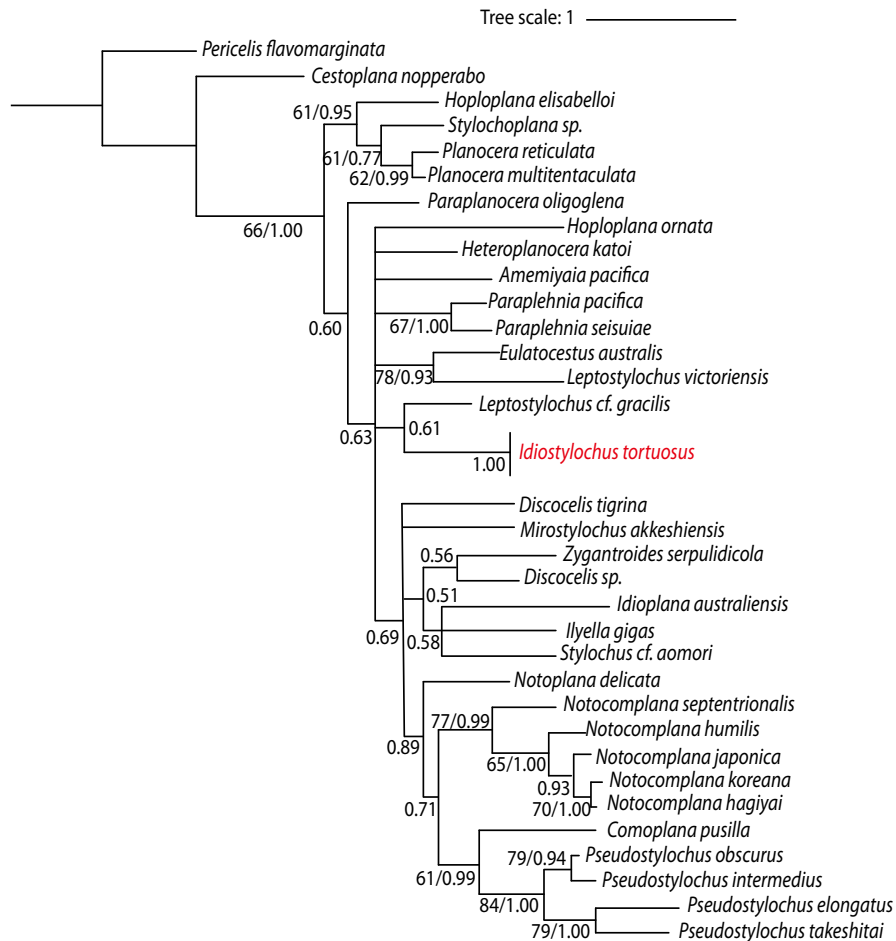


Fig. 5. Maximum likelihood / Bayesian Inference tree based on partial sequences of the *COI* gene (total length: 640 bp). Numbers in the nodes correspond to the bootstrap support values of the ML analysis (> 60) and the posterior probability values of the BI analysis (> 0.50), respectively.

in the Family Idioplanidae, diagnosed now as:

Stylochoidea with or without tentacles; male copulatory organ with seminal vesicle or spermiducal bulbs. Unarmed penis; vasa deferentia or spermiducal bulbs unite before entering into the copulatory organ. Female copulatory organ developed with vagina bulbosa, abundant cement and shell glands and long vagina interna.

After COI analysis, *Idiostylochus* are closely related to *Leptostylochus*. However, in the 28S analysis, both genera appear separated into two different families. *Idiostylochus* was included in the family Idioplanidae, and *Leptostylochus* was included in the family Latocestidae, along with *Mirostylochus* and *Latocestus* (0.96) (Dittmann et al. 2019; Litvaitis et al. 2019; Oya and Kajihara 2020; Rodriguez et al. 2021).

Morphologically, *Leptostylochus* and *Mirostylochus*, clustered with *Latocestus* (Fig. 4), as they presented the external features of Latocestidae (absence of tentacles, cuneiform body, eyes scattered between frontal margin and brain). Within the anatomical characters, the free prostatic vesicle and the seminal receptacle developed as a false seminal vesicle or spermiducal bulb, but not as true seminal vesicle, are characteristic of the Latocestidae and not of the

Stylochidae, the former family of *Leptostylochus* and *Mirostylochus* (Bock 1925; Kato 1937).

CONCLUSIONS

Idiostylochus tortuosus as well as other genera of polyclad flatworms like *Stylochus*, are predators of mollusks of economic interest and can be a serious threat to aquaculture, potentially expanding to where these species are being exploited (Danglade 1919; Galleni et al. 1980; Sluys et al. 2005; Gammoudi et al. 2017) (Fig 6). For this reason, it is recommended that control measures be implemented as soon as possible to reduce the impact on bivalve exploitation and prevent its possible expansion along the European Atlantic Coast. It is necessary to test the efficacy of nonspecific control methods such as hyper- and hyposaline baths (Espinosa 1981; O'Connor and Newman 2001), calcium hypochlorite solutions (Yang 1974), and the removal of fouling cover (Littlewood and Marsbe 1990). These control methods have already been used to combat other species of polyclad flatworms and could be effective in the control of this new species.

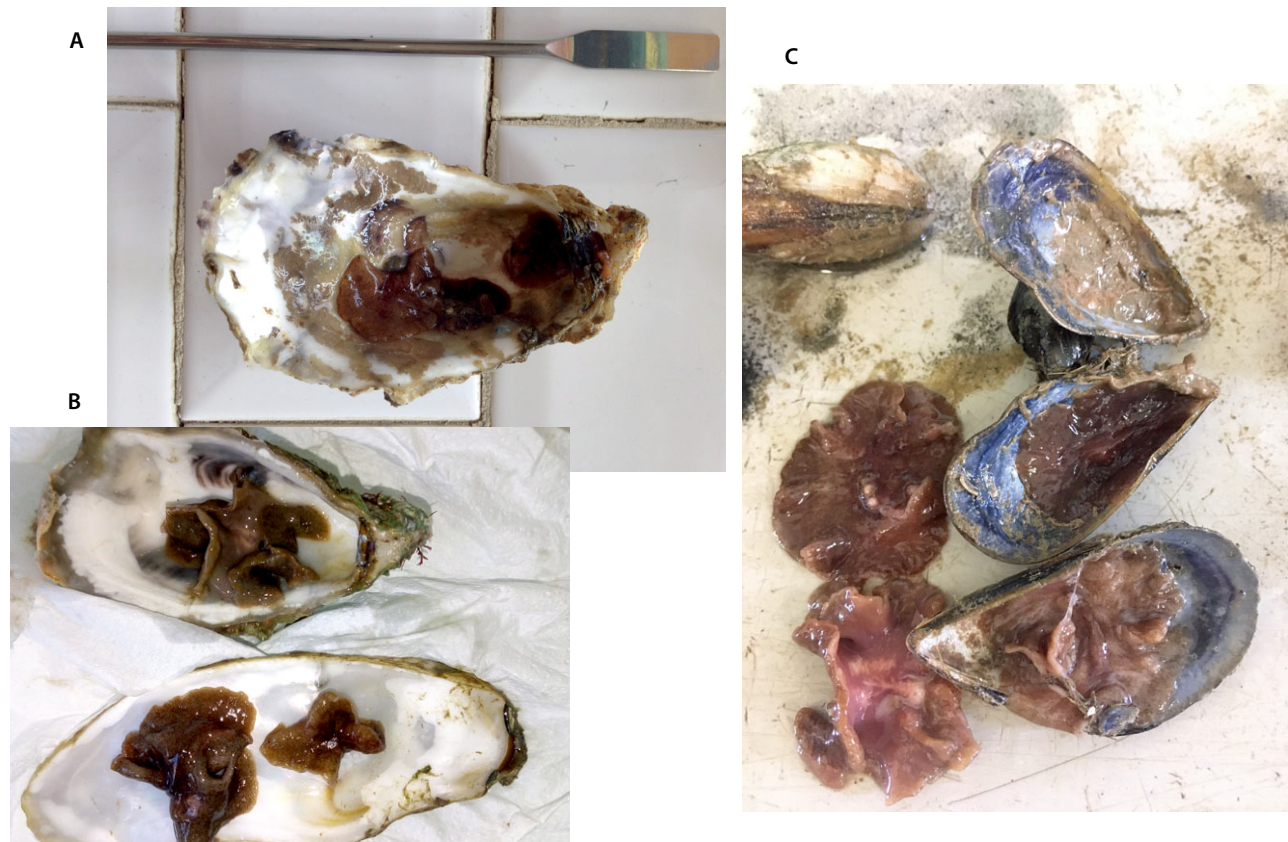


Fig. 6. *Idiostylochus tortuosus* photographed A–B, inside oysters (Bay d'Arcachon) and C, inside mussels (Biarritz).

List of abbreviations

fp, female pore.
 mp, male pore.
 op, oral pore.
 ovd, oviduct.
 ph, pharynx.
 spb, spermiducal bulbs.
 shg, shell glands.
 vd, vasa deferentia.
 ve, vagina externa.
 vi, vagina interna.
 Lv, Lang's vesicle.
 pv, prostatic vesicle.
 sv, seminal vesicle.

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Availability of data and materials: The data and information used in this manuscript is available as follows: *COI* and 28S sequences have been submitted to NCBI-GenBank as reflected in table 1. Histologically processed individuals can be consulted in the collections of the National Museum of Natural Sciences (MNCN-CSIC) through the corresponding catalog number, as it appears in the "Material studied" in the description of the species. Other electronic information has not been generated for this manuscript.

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